AD_____

Award Number: W81XWH-04-1-0104

TITLE: The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation and Dysfunctional Inflammation in a Swine Grade V Liver Injury Model

PRINCIPAL INVESTIGATOR: Martin A. Schreiber, MD

CONTRACTING ORGANIZATION:

Oregon Health & Science University
Portland, Oregon 97239

REPORT DATE: January 2011

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

    X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-01-2011	Final	8 DEC 2003 - 7 DEC 2010

4. TITLE AND SUBTITLE

The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation and Dysfunctional Inflammation in a Swine Grade V Liver Injury Model

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0104

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Martin A. Schreiber, Jerome Differding, Philbert Gregory Hamilton, Nicholas Kunio, Ross Anderson

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Oregon Health & Science University
Portland, Oregon 97239

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT *(Maximum 200 Words) (abstract should contain no proprietary or confidential information)*

Purpose: To determine the efficacy of an initial bolus of resuscitative fluids currently utilized in military and civilian settings on the physiologic response to uncontrolled hemorrhage. **Scope:** 50 swine underwent A grade V liver injury was performed, followed by 30 minutes (30') of uncontrolled hemorrhagic shock. After 30', liver packing was completed and randomized blinded fluid resuscitation was initiated over a 12' period with two liters of normal saline (NS), two liters of Lactated Ringer's (LR), 250 ml of 7.5% saline with 3% Dextran (HTS), 500 ml of Hextend (HEX), or no fluid (NF). Animals were monitored for 2 hours post injury. Physiologic parameters, coagulation assays and inflammatory mediators were compared. **Major Findings:** The NF group had less post-treatment blood loss compared to the fluid groups. MAP and StO_2 for HEX, HTS, and LR at 1 HR and 2 HR were comparable and superior to NF. NS was not statistically different from NF for MAP and StO_2 but did result in lower pH and decreased base excess.

15. SUBJECT TERMS

Uncontrolled hemorrhage, resuscitation, animal model, coagulopathy, swine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU		USAMRMC
U	U	U		22	**19b. TELEPHONE NUMBER**

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION:

Each year, trauma is responsible for 37 million emergency department visits and 2.6 million hospitalizations. Hemorrhage accounts for 30 to 40% of all trauma deaths and is the most common cause of preventable death after injury. Fluid resuscitation remains a critical element of the early treatment of trauma patients, but the optimal fluid strategy remains highly debated.

Since the early 1980's, generations of physicians and emergency personnel have been trained according to Advanced Trauma Life Support (ATLS) principles. The ATLS course advocates utilization of isotonic electrolyte solutions such as Lactated Ringer's (LR) or normal saline (NS). These guidelines promote an initial fluid bolus of 1 to 2 liters of isotonic crystalloids should be given, and then further diagnostic and therapeutic decisions are based on the observed response to the initial fluid challenge.

The current protocol in the United States military as recommended by the Committee on Tactical Combat Casualty Care is to utilize a palpable radial pulse and normal mentation as primary endpoints to either start or stop fluid resuscitation. If a warfighter suffers a significant injury with or without blood loss and maintains a normal mental status and a palpable radial pulse, medics are instructed to obtain IV access and withhold fluid, re-evaluating the casualty as often as the situation allows. However, if the casualty experiences significant blood loss and either diminished mental status or a loss of a palpable radial pulse, then care providers are educated to utilize hemorrhage control techniques, and initiate a 500 ml bolus of Hextend (one bag) until a palpable radial pulse returns or mental status improves. The maximum dose of Hextend that is currently recommended for field resuscitation is one liter.

The Eastern Association for the Surgery of Trauma (EAST) has developed guidelines for the initial resuscitation of trauma patients based on a structured evidence-based review of the literature. This group concluded that, similar to military protocol, in patients with penetrating injury and short transport times, fluids should be withheld for patients with a palpable radial pulse or normal mental status until they reach definitive care. If fluid is given, the consensus group noted that there was insufficient evidence to recommend one type of fluid over another. However, the authors noted that small volume (250 ml) boluses of hypertonic solutions seemed equivalent to larger boluses of crystalloid (1000 ml) with respect to hemodynamic response and volume expansion.

Given that the ideal fluid for initial resuscitation has not been elucidated and there is an observed discrepancy in current guidelines, the objective of the current study was to determine the efficacy of an initial bolus of resuscitative fluids currently utilized in the military and civilian settings on the physiologic response to uncontrolled hemorrhagic shock in a swine Grade V liver injury model. Based on prior animal studies within our laboratory, we hypothesized that bolus resuscitation with NS would result in inferior hemodynamic and physiologic outcomes compared to other commonly used resuscitation fluids.

BODY:

Materials and Methods

Specific Aim 6 Materials – Advanced Resuscitation Strategies (<u>Starting Analysis</u>)

The model was developed at the Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee.

Female Yorkshire Crossbred swine underwent the following polytrauma protocol to assess the efficacy of Tranexamic acid (TXA). This drug is commonly prescribed for control of excess bleeding. To date only a single trial in trauma (CRASH-2) has been conducted. This study does not answer the mechanistic questions in relation to controlling hemorrhage. We were hoping to identify that with this project.

Specific Aim 6 Methods – Efficacy of Tranexamic Acid in a Model of Polytrauma

Nineteen swine (12 study, 6 model development, 1 death) were utilized for this experiment. Animals were delivered 7 to 10 days prior to the experiment in order to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. Animals were fasted for 16 hours the day before surgery. Water was available ad libitum. A single vendor was used to eliminate potential differences in animal strain.

Anesthesia

On the day of the experiment animals were given an induction agent consisting of 8 mg/kg Telazol® (tiletamine hydrochloride 50 mg/ml, zolazepam hydrochloride 50 mg/ml, Fort Dodge Animal Health, Fort Dodge, Iowa) given intramuscularly. Animals were placed in the supine position. Orotracheal intubation was performed and a 7.5mm internal diameter cuffed endotracheal tube was placed. The endotracheal tube was connected to the anesthesia machine with 1-3% isoflurane for anesthetic maintenance in 50% oxygen. Tidal volume was fixed at 10 ml/kg with a rate of 10 breaths per minute. An esophageal stethoscope, gastric tube and thermometer were inserted. An EKG monitor was secured and continuous monitoring started. Throughout the study, anesthesia was maintained to the clinical endpoints of reflexes and muscle relaxation as is done in humans.

Monitoring, access and pre-experiment procedures

After swine were anesthetized a left cervical cutdown was performed and polyethylene catheters were inserted respectively into the left common carotid and left external jugular vein. The arterial line was utilized for the controlled hemorrhage and blood sampling throughout the experiment while the venous line was used for administration of bolus resuscitation fluids and TXA. Finally, a proximal femoral cutdown was performed and the artery was cannulated for continuous blood pressure monitoring. Mean arterial pressure (MAP) was continuously recorded and averaged every 10 seconds with a blood pressure analyzer and digital data collection system (DigiMed, Louisville, KY). Baseline labs were collected and included electrolytes, lactate, spun hematocrit (Hct), activated clotting time (ACT), platelets (Plt), prothrombin time (PT), partial thromboplastin time (PTT), and arterial blood gas (ABG) and additional coagulation factors. In addition, a baseline thrombelastogram (TEG, Haemoscope Corporation, Niles, IL) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Injury Phase

After needle localization, a captive bolt gun was used to fracture the femur and create a soft tissue injury at the midshaft of the left femur. A controlled hemorrhage was then initiated to remove 50% of the blood volume based on a published, standard equation relating blood volume to body weight for domestic swine. During hemorrhage if the mean arterial blood pressure (MAP) fell below 25mm/Hg, normal saline (NS) was infused at a rate of 165 ml/min to keep the MAP>25 mm/Hg. The animal was also cooled to 33 +/-0.4^{o}C using cooled intraperitoneal lavage with crystalloid as needed (most of the animals developed a degree of hypothermia spontaneously due to shock and infusion of IV fluids). These procedures were followed by a 30-minute shock period, representing time in the field prior to medical intervention.

Prehospital care/transport phase

After the 30-minute shock period, electrolytes, spun hematocrit, ACT, PT, PTT, platelets, ABG, factors and TEG were again recorded. After coagulation studies and lab collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 ml/min, minus any given during the controlled hemorrhage. This reflects current civilian pre-hospital resuscitative practices. At the same time as the replacement fluid infusion, the TXA or the control (Normal Saline) drip is initiated at a bolus of 25 cc over 10 minutes. After the initial 10-minute bolus, a drip is continued over the next 4 hours and 26 minutes of 13.83 cc.
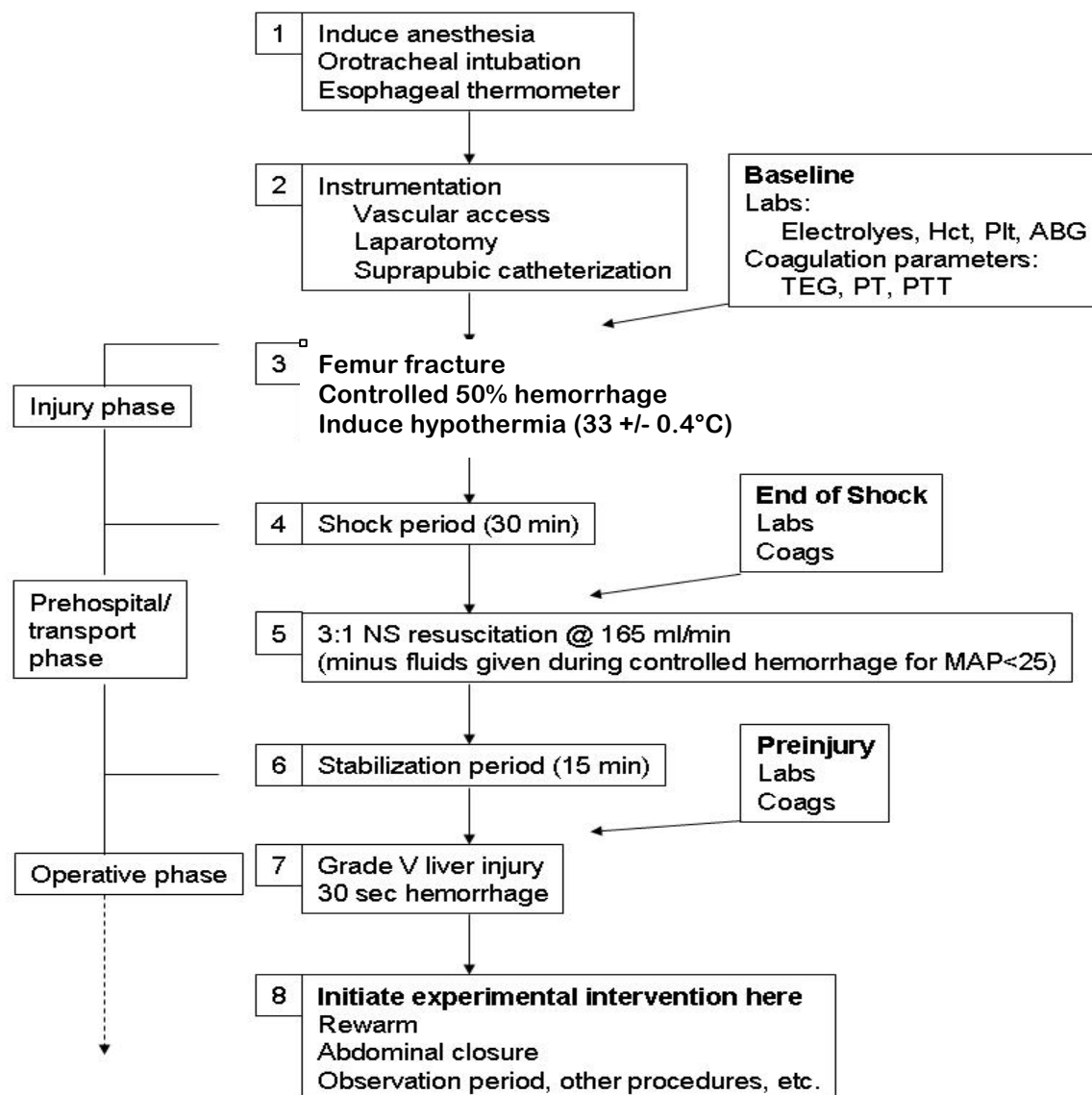
Operative phase

Following NS resuscitation, a 15-minute stabilization period was observed; during which a baseline MAP was recorded and pre-weighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. Labs and coagulation studies were again collected, and a previously described grade V liver injury was created at the confluence of the right and middle hepatic veins using a specialized clamp.

Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen and packing of the liver with a fixed number of additional pre-weighed laparotomy sponges. The liver injury was designed to provide a second stressor after initial injury and also to create a standardized injury that had the potential to re-bleed, both of which simulate a laparotomy after trauma in a patient with solid organ injury. Thirty seconds after injury, the liver was packed with laparotomy sponges in a standardized fashion. The animal was also re-warmed to 37°C and the abdomen closed with towel clips. These animals received no additional fluid replacement after the liver injury.

Follow-up

Animals were monitored for 4 hours post injury or to death. Labs were collected at 1, 2, 3 and 4 hours. If the MAP fell below 15 mmHg it was denoted as death and the time of death was recorded. Animals surviving 4 hours were euthanized with Euthasol.

Lung tissue was collected at the end of 4 hours or at declaration of death for rtPCR analysis. Tissue was stored in RNA later and a 10% buffered formalin solution. An autopsy of the liver was performed to ensure comparable injuries without portal vein involvement.

Induce anesthesia
1 | Induce anesthesia
Orotracheal intubation
Esophageal thermometer

2 | Instrumentation
   Vascular access
   Laparotomy
   Suprapubic catheterization

Baseline
Labs:
   Electrolyes, Hct, Plt, ABG
Coagulation parameters:
   TEG, PT, PTT

Injury phase

3 | Femur fracture
Controlled 50% hemorrhage
Induce hypothermia (33 +/- 0.4°C)

4 | Shock period (30 min)

End of Shock
Labs
Coags

Prehospital/
transport
phase

5 | 3:1 NS resuscitation @ 165 ml/min
(minus fluids given during controlled hemorrhage for MAP<25)

6 | Stabilization period (15 min)

Preinjury
Labs
Coags

Operative phase

7 | Grade V liver injury
30 sec hemorrhage

8 | **Initiate experimental intervention here**
Rewarm
Abdominal closure
Observation period, other procedures, etc.

Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Laboratory values include Hct, lactate, Plt, ABG, and electrolytes. Coagulation parameters include the PT, PTT, ACT, and Factors II, V, VII, VIII, IX, X, XI, XII, Protein C, Protein S

Statistical Analysis

Analysis of this project is just beginning. The study was terminated prior to completion due to premature death of all animals prior to the 4-hour time point. Study variables are still being analyzed to see if there are differences in the study animals (TXA) or controls (normal saline).

Specific Aim 8 Materials – Comparison of Current Military Resuscitation Fluids

The model was developed at the Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee.

Female Yorkshire Crossbred swine underwent the following protocol to determine the efficacy of an initial bolus of resuscitative fluids currently utilized in military and civilian settings on physiologic response to uncontrolled hemorrhagic shock. It was hypothesized that resuscitation with normal saline would result in inferior outcomes compared to other commonly used resuscitation fluids.

An InSpectra Tissue Spectrometer (Hutchinson Technology, Hutchinson, MN) was utilized for the monitoring of tissue oxygen saturation (StO_2). Physiological parameters including blood pressure, heart rate and mean arterial pressure were monitored using a continuous blood pressure monitor (DigiMed, Louisville, KY).

Specific Aim 8 Methods – Initial Resuscitation in Uncontrolled Hemorrhagic Shock

This study was designed as a prospective, randomized, blinded trial in a swine model that took place in a Level 1 Trauma Center animal laboratory. The Institutional Animal Care and Use Committee at Oregon Health & Science University approved the protocol. This facility adheres to the National Institutes of Health guidelines for the care and use of laboratory animals.

Fifty similarly sized Yorkshire-crossbred female swine underwent a 16 hour pre-operative fast except for water ad libatum. The swine were pre-anesthetized with an intramuscular injection of 8 mg/kg Telazol (Fort Dodge Animal Health, Fort Dodge, IA) and then intubated orally with a 7.0 to 7.5 mm endotracheal tube. Animals were placed on mechanical ventilation and constantly monitored by an independent animal technician who adjusted the respiratory rate to maintain the pCO_2 between 40 to 50 mm Hg. Anesthesia was maintained with 2% isoflurane in 100% oxygen with the animal technician monitoring jaw laxity to assess anesthesia adequacy. An esophageal monitor was placed and euthermia was obtained utilizing external warming devices.

After swine were anesthetized, additional monitoring devices were placed. An InSpectra Tissue Spectrometer (Hutchinson Technology, Hutchinson, MN) was placed on the left hind limb of the swine for continuous monitoring of tissue oxygen saturation (StO_2) throughout the experiment. Left cervical cutdowns were performed, and polyethylene catheters were inserted respectively into the left common carotid and left external jugular vein. The arterial line was utilized for continuous blood pressure monitoring, and mean arterial pressure (MAP) was continuously recorded and averaged every 10 seconds with a blood pressure analyzer and digital data collection system (DigiMed, Louisville, KY). The venous line was used for administration of bolus resuscitation fluids. Finally, a left femoral cutdown was performed, and the left femoral artery was cannulated for blood sampling throughout the experiment.

After placement of these monitoring devices, a midline celiotomy and suprapubic Foley catheterization were performed. Following a 15-minute stabilization period, residual peritoneal fluid was removed from the abdominal cavity. Pre-weighed laparotomy sponges were placed in the left and right pericolic gutters as well as the pelvis to facilitate primary hemorrhage collection. Then, a standardized Grade V liver injury (injury to a central hepatic vein, consistent with the AAST scaling and scoring system) was created using a specially designed clamp with 4 razor sharp edges (Figure 1). This protocol for liver injury has been previously validated in our

prior studies of uncontrolled hemorrhagic shock. The time of injury was considered the start time for the 2 hour experimental time period.

Animals were allowed to hemorrhage for 30 minutes during which time the primary blood loss was collected with wall suction and by the three previously placed pre-weighed laparotomy sponges. After 30 minutes, the three laparotomy sponges were removed, and the liver was then packed with six pre-weighed laparotomy sponges in a manner consistent with a damage control operation in order to collect secondary blood loss. The abdomen was then temporarily closed with penetrating towel clamps.

Animals were randomized to 4 different groups for bolus fluid resuscitation, which was initiated 30 minutes after injury. The fluids administered included fluids and associated volumes currently utilized for bolus resuscitation in both civilian and military practice as follows: 2 liters of NS, 2 liters of LR, 500 ml of Hextend (HEX), and 250 ml of 7.5% hypertonic saline with 3% Dextran (HTS). A no fluid (NF) arm acted as a control group. Ten animals were randomized to each respective fluid/control group. Surgical staff was blinded to fluid resuscitation, as all fluids were administered over a 12-minute time period from a sterile opaque container which was previously filled by an independent technician who controlled fluid rate ml/min to achieve resuscitation in the 12 minute allotted time period regardless of fluid type. Surgical staff was only aware of the control/no fluid group randomization after creation of the standardized injury and temporary closure of the abdominal cavity.

The animals that did not die from exsanguination during the course of the experiment were sacrificed at the completion of the 2-hour study with a euthanasia solution. Then, the six pre-weighed laparotomy sponges placed prior to abdominal closure were removed and weighed to determine secondary blood loss. Lung tissue was collected at the end of 2 hours or at declaration of death for rtPCR analysis. Tissue was stored in RNA later and a 10% buffered formalin solution. An autopsy of the liver was performed to ensure comparable injuries without portal vein involvement.

Study Variables

Physiologic variables included survival, MAP, StO_2, blood loss from the uncontrolled hemorrhage, and blood loss post liver injury. Laboratory values include Hct, lactate, ABG, chemistry panel, and complete blood count (CBC). Coagulation parameters include the PT, PTT, ACT, and Factors II, V, VII, VIII, IX, X, XI, XII, Protein C, Protein S. Serum was collected for analysis of inflammatory cytokines at time points baseline, one-hour and end of study.

Statistical Analysis

Analysis of variance (ANOVA) was used to compare values within a group utilizing statistical package software SPSS, version 18.0 (SPSS Inc., Chicago, IL). Student's *t* test was used to compare means for parametric, normally distributed data as mean ± SEM, while the Mann-Whitney test was used to compare non-normally distributed data as median with IQR. Comparisons within groups utilized a paired *t* test for normally distributed data, and non-parametric data was assessed using the Kruskal-Wallis test. Statistical significance was defined as a *p* value < 0.05.

Results

Physiologic and Laboratory Analysis

Ten animals were randomized to each group. The baseline weight of swine in all groups was similar (Table 1). Two animals in the NF group did not survive to study completion; however, their data is included in the analysis up until the time of their deaths. There was no statistically significant difference in survival between groups, $p = 0.50$. All animals had similar Grade V liver injuries without portal vein injury as determined by autopsy. Primary blood loss before resuscitation was similar for all groups (Table 1).

Secondary blood loss after resuscitation is presented in Table 1. The secondary blood loss in the NF group was significantly lower than all other groups, $p < 0.01$. Though the NS and LR groups had similar secondary blood losses, which were higher than both the HEX and HTS groups, none of these groups were statistically different from one another.

Laboratory values for hematocrit (Hct) are presented in Table 2. At both the 1 and 2-hour time points, the NF group had significantly higher Hcts compared to the other groups, $p < 0.01$. The LR group had a significantly higher Hct compared to the NS, HEX, and HTS groups at 1 hour and compared to the HEX and HTS groups at 2 hours, all $p < 0.03$. Additionally, the NS group had a significantly higher Hct compared to the HEX group at 2 hours, $p < 0.02$.

Continuous MAP data for all groups is presented in Figure 2. All animals experienced a significant initial drop in MAP during the uncontrolled hemorrhage period, followed by a period of spontaneous blood pressure elevation. At 30 minutes, bolus fluid resuscitation resulted in a significant increase in MAP, after which, the MAP decreased over the rest of the study period. The observed increase in MAP in the NF group at 70 and 75 minutes was secondary to the death of animals in this group at these two time points.

MAP data at baseline, 1 hour, and 2 hours are presented in Table 3. At 1 hour, both the NS group and the NF group had a significantly lower MAP compared to the LR, HEX, and HTS groups, all $p < 0.05$. At 2 hours, the MAP in the NS group was significantly lower than the LR, HEX, and HTS groups, all $p < 0.04$. The MAP did not differ between the LR, HEX, and HTS groups at 1 or 2 hours. The MAP in the NF group was not different from the other groups at 2 hours.

Continuous StO_2 data for all groups is presented in Figure 3, with a similar curve as previously noted with MAP. StO_2 data at baseline, 1 hour, and 2 hours is presented in Table 3. At 1 hour, the NF group had a significantly lower StO_2 compared to the other groups, $p < 0.04$. At 2 hours, the StO_2 in the NF group was lower than the LR, HEX, and HTS groups, all $p < 0.04$. Also at 2 hours, the StO_2 in the NS group was lower than the HTS group, $p = 0.02$. There was no difference between the NF and NS groups at 2 hours. StO_2 did not differ between the LR, HEX, and HTS groups at 1 hour or 2 hours.

Laboratory values for pH are presented in Table 4. Swine are alkalotic at baseline, which has been observed in prior experiments. At 1 hour, there was no difference between groups. At 2 hours, the NS group had a significantly lower pH compared to the LR, HEX, and NF groups, all $p < 0.02$. Also at 2 hours, the HTS group had a significantly lower pH compared to the LR and HEX groups, all $p < 0.05$.

Laboratory values for base excess are shown in Figure 4. The NS group had a significantly lower base excess compared to the LR group at 1 hour and 2 hours, the HEX group at all time points, and the NF group at 2 hours, all $p < 0.03$. The HTS group had lower base excess than the HEX group at 1 hour and the LR, HEX, and NF groups at 2 hours, all $p < 0.01$.

The LR group had a significantly lower base excess compared to HEX at all time points, all $p < 0.03$.

Lactate values are also shown in Table 4. All groups demonstrated a significant increase in lactate from baseline to 1 hour, all $p < 0.01$. At 2 hours, there was a significant decrease in lactate levels from the 1 hour time point in the LR, HEX, HTS, and NF groups, all $p < 0.04$. However, there was no significant decrease from 1 hour to 2 hours in the NS group, $p = 0.14$. There were no significant differences between fluid groups at any time point.

Coagulation and Inflammatory Markers

Analysis of both the coagulation parameters and inflammatory markers are currently ongoing.

KEY RESEARCH ACCOMPLISHMENTS – Specific Aim 8

1. Baseline characteristics similar
2. Animals receiving NF had less secondary blood loss
3. 1 hour post injury MAP for LR, HEX and HTS is significantly better than NS or NF
4. 2 hour post injury MAP only NS was significantly lower / worse than all other fluids
5. HEX provides more consistent StO_2 compared to other fluids
6. In clinically utilized bolus volumes, HEX, HTS and LR are similar in all study with respect to all study parameters
7. 250 ml bolus of HTS provides equivalent resuscitation to the larger dosing regimens but results in a more relatively hypocoagulable state
8. NF provides a similar outcome to NS with a less acidotic state and decreased secondary blood loss. This makes one question if NS should be utilized as an initial fluid resuscitation.

REPORTABLE OUTCOMES

Specific Aim 6

Once this data is completely analyzed appropriate findings will be sent as initial abstracts to National meetings for presentation with manuscripts following.

Specific Aim 8

This data was presented at the Region X Committee on Trauma Competition. This data was awarded first prize at the Oregon chapter of Society of Critical Care Medicine. A presentation was given at this meeting in Vancouver, WA. In addition, an abstract was submitted and accepted to the 2011 annual meeting of Pacific Coast Surgical Association in Scottsdale, AZ. It was also selected to compete for the Resident competition award at that meeting. A manuscript has been generated and submitted for this competition to Archives of Surgery.

BIBLIOGRAPHY OF PUBLISHED WORK OVER THE COURSE OF THE GRANT

MANUSCRIPTS

1. Watters JM, Brundage SI, Todd SR, Zautke NA, Stefater JA, Lam JC, Muller PJ, Malinoski D, **Schreiber MA**. Resuscitation with Lactated Ringer's Does Not Increase Inflammatory Response in a Swine Model of Uncontrolled Hemorrhagic Shock. Shock. 2004; 22:283-287.

2. Brundage SI, Zautke NA, Watters JM, Stefater JA, Todd SR, Lam JC, **Schreiber MA.** Lactated Ringer's Does Not Increase Inflammation after Shock. 6th World Congress on Trauma, Shock, Inflammation and Sepsis. 2004; EC302C0210:151-155.

3. Todd SR, Malinoski DJ, Muller PJ, **Schreiber MA**. Hextend Attenuates Hypercoagulability Following Severe Injury in Swine. Journal of Trauma. 2005:59;589-594.

4. Kiraly LN, Differding JA, Enomoto TM, Sawai RS, Muller PJ, Diggs B, Tieu BH, Englehart MS, Underwood S, Wiesberg TT, **Schreiber MA**. Resuscitation with Normal Saline (NS) vs. Lactated Ringers Modulates Hypercoagulability and Leads to Increased Blood Loss in an Uncontrolled Hemorrhagic Shock Swine Model. Journal of Trauma. 2006:61;57-65.

5. Watters JM, Tieu BH, Differding JA, Muller PJ, **Schreiber MA**. A Single Bolus of 3% Hypertonic Saline with 6% Dextran Provides Optimal Initial Resuscitation after Uncontrolled Hemorrhagic Shock. Journal of Trauma. 2006:61;75-81.

6. Watters JM, Tieu BH, Todd SR, Jackson T, Muller PJ, Malinoski DJ, **Schreiber MA.** Fluid Resuscitation Increases Inflammatory Gene Transcription Following Traumatic Injury. Journal of Trauma. 2006;61;300-309.

7. Todd SR, Malinoski DJ, Muller PJ, **Schreiber MA**. Lactated Ringer's is Superior to Normal Saline in the Resuscitation of Hemorrhagic Shock. Journal of Trauma. 2007;62:636-639.

8. Tieu BH, Holcomb JB, **Schreiber MA**. Coagulopathy: Its Pathophysiology and Treatment in the Injured Patient. World Journal of Surgery. 2007;31:1055-1065.

9. Englehart MS, AlTieu BH, Kiraly LN, Underwood SA, Muller PJ, Sawai RS, **Schreiber MA**. Ketamine-Based Total Intravenous Anesthesia is Superior to Isoflurane in a Swine Model of Hemorrhagic Shock. Journal of Trauma. 2008 Oct;65(4): 901-8; discussion 908-09.

10. Cho SD, Holcomb JB, Tieu BH, Englehart MS, Morris MS, Karahan ZA, Underwood SA, Muller PJ, Prince MD, Medina L, Sondeen J, Shults C, Duggan M, Tabbara M, Alam HB, **Schreiber MA**. Reproducibility of An Animal Model Simulating Complex, Combat-Related Injury in a Multiple-Institution Format. Shock. 2009 Jan;31 (1): 87-96.

11. Spoerke N, Zink K, Cho SD, Differding J, Muller P, Karahan A, Sondeen J, Holcomb JB, Schreiber M. Lyophilized Plasma for Resuscitation in a Swine Model of Severe Injury. Archives of Surgery. 2009 Sep;144(9):829-834.

12. Alam HB, Bice LM, Butt MU, Cho SD, Dubick MA, Duggan M, Englehart MS, Holcomb JB, Morris MS, Prince MD, Schreiber MA, Shults C, Sondeen JL, Tabbara M, Tieu BH, Underwood SA; Hemostatic Resuscitation Research Group. Testing of blood products in a polytrauma model: results of a multi-institutional randomized preclinical trial. Journal of Trauma. 2009 Oct;67(4):856-64.

13. Spoerke NJ, Van PY, Differding JA, Zink KA, Cho SD, Muller PJ, Karahan ZA, Sondeen JL, Holcomb JB, Schreiber MA. Red blood cells accelerate the onset of clot formation in polytrauma and hemorrhagic shock. Journal of Trauma. 2010 Nov;69(5):1054-1059; discussion 1059-61.

ABSTRACTS

1. Todd SR, Malinoski D, **Schreiber MA**. Lactated Ringer's is Superior to Normal Saline in Uncontrolled Hemorrhagic Shock. Shock. 2003;Supplement to Volume 19:169.

2. Todd SR, Malinoski D, **Schreiber MA**. Hextend Attenuates the Hypercoagulability Following Severe Liver Injury in Swine. Journal of Trauma. 2004;56:226.

3. Stefater, Brundage SI, Watters J, Zautke N, Todd SR, Lam, **Schreiber MA.** Lacated Ringer's does not Increase Inflammation after Shock. Shock 2004;Supplement to Volume 21:458.

4. Differding JA, Watters JM, Muller PJ, **Schreiber MA**. The Correlation between Mean Arterial Blood Pressure and Tissue Oxygenation after Uncontrolled Hemorrhagic Shock. Journal of Surgical Research. 2004;126:336-337.

5. Watters JM, Jackson T, Muller PJ, Malinoski D, Todd SR, **Schreiber MA**. Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury. Journal of Trauma. 2004;57:1378.

6. Watters JM, Differding JA, Muller PJ, **Schreiber MA**. A Single Bolus of 3% Hypertonic Saline with Dextran Provides Optimal Resuscitation after Uncontrolled Hemorrhagic Shock. Journal of Trauma. 2005;58:216.

7. Wiesberg TT, Differding JA, Enomoto TM, Sawai RS, Muller PJ, **Schreiber MA**. Resuscitation with Lactated Ringer's (LR) vs. Normal Saline (NS) Leads to a Hypercoagulable State in an Uncontrolled Hemorrhagic Shock Swine Model. Journal of Trauma. 2006;59:1538.

8. Englehart M, Tieu B, Kiraly L, Sawai R, **Schreiber MA**. The Influence of Anesthesia on a Swine Model of Hemorrhagic Shock. Shock. 2006;25:58.

9. Tieu B, Enomoto TM, Phillips C, Hagg D, Wiesberg T, Muller P, **Schreiber MA**. Resuscitation with Normal Saline (NS) Increased Volume Requirement, Increased Cardiac Output and Decreased Systemic Vascular Resistance compared to Lactated Ringers (LR) in an Uncontrolled Hemorrhagic Shock Model in Swine. Journal of the American College of Surgeons. 2006;32:S32.

10. Kiraly LN, Sawai RS, Watters JM, Differding JA, Wiesberg TT, Muller PJ, **Schreiber MA**. 7.5% Saline with Dextran Resuscitation Causes Dysfunctional Inflammation in Uncontrolled Hemorrhagic Shock. Journal of Trauma. 2006;61:1559.

11. Englehart MS, Kiraly LN, Tieu BH, Sawai RS, Underwood SA, Muller PJ, **Schreiber MA**. Ketamine-Based Total Intravenous Anesthesia (TIVA) is Superior to Isoflurane (ISO) in a Swine Model of Hemorrhagic Shock. Journal of Trauma. 2006;61:1559.

12. Sondeen J, Shults C, Holcomb J, Alam H, **Schreiber MA**. Reproducibility of a Complex Hemorrhage Shock Model and Tissue Injury in Swine at Three Academic Research Centers. Shock. 2007;27:74.

Figure 1. Creation of a standardized Grade V liver injury in the central portion of the liver with a specialized clamp that has 4 sharpened edges.
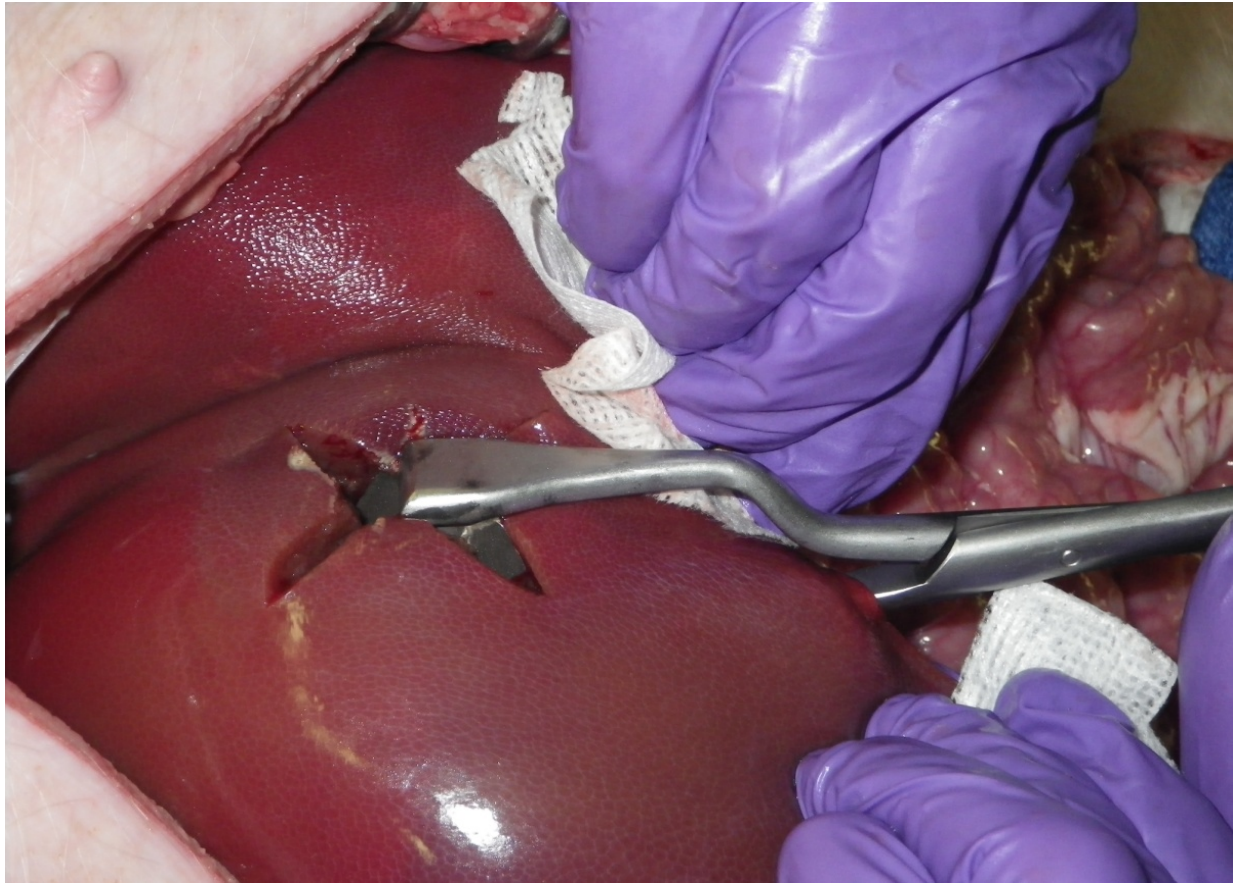
Figure 2. Continuous Mean Arterial Pressure (MAP).

Data presented are the average MAP for each group at each specific time point. Grade V liver injury was created at time point 0. Bolus fluid administration began at time point 30 min.
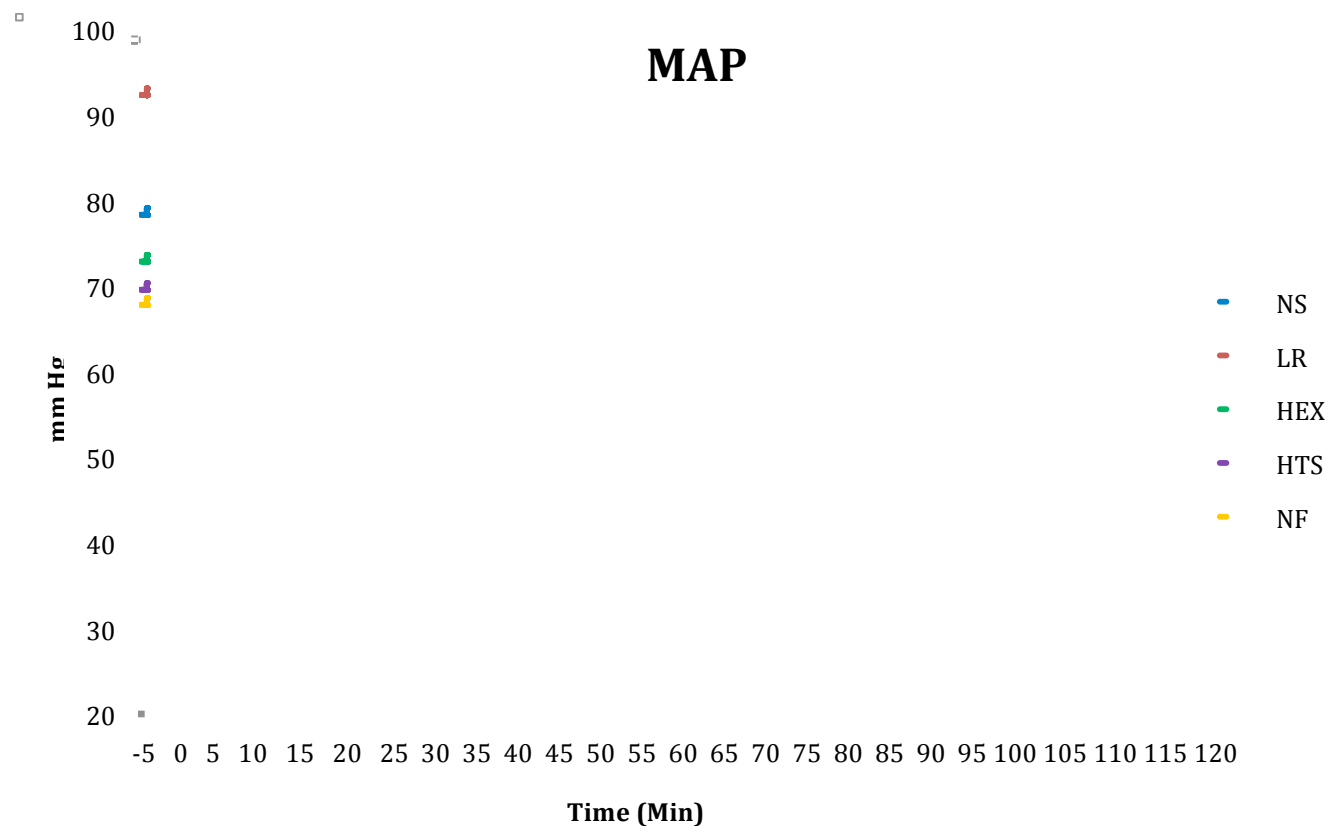

MAP

mm Hg

NS
LR
HEX
HTS
NF

Time (Min)

Figure 3. Continuous tissue oxygen saturation (StO$_2$).

Data presented are the average StO$_2$ for each group at each specific time point. Grade V liver injury was created at time point 0. Bolus fluid administration began at time point 30 min.
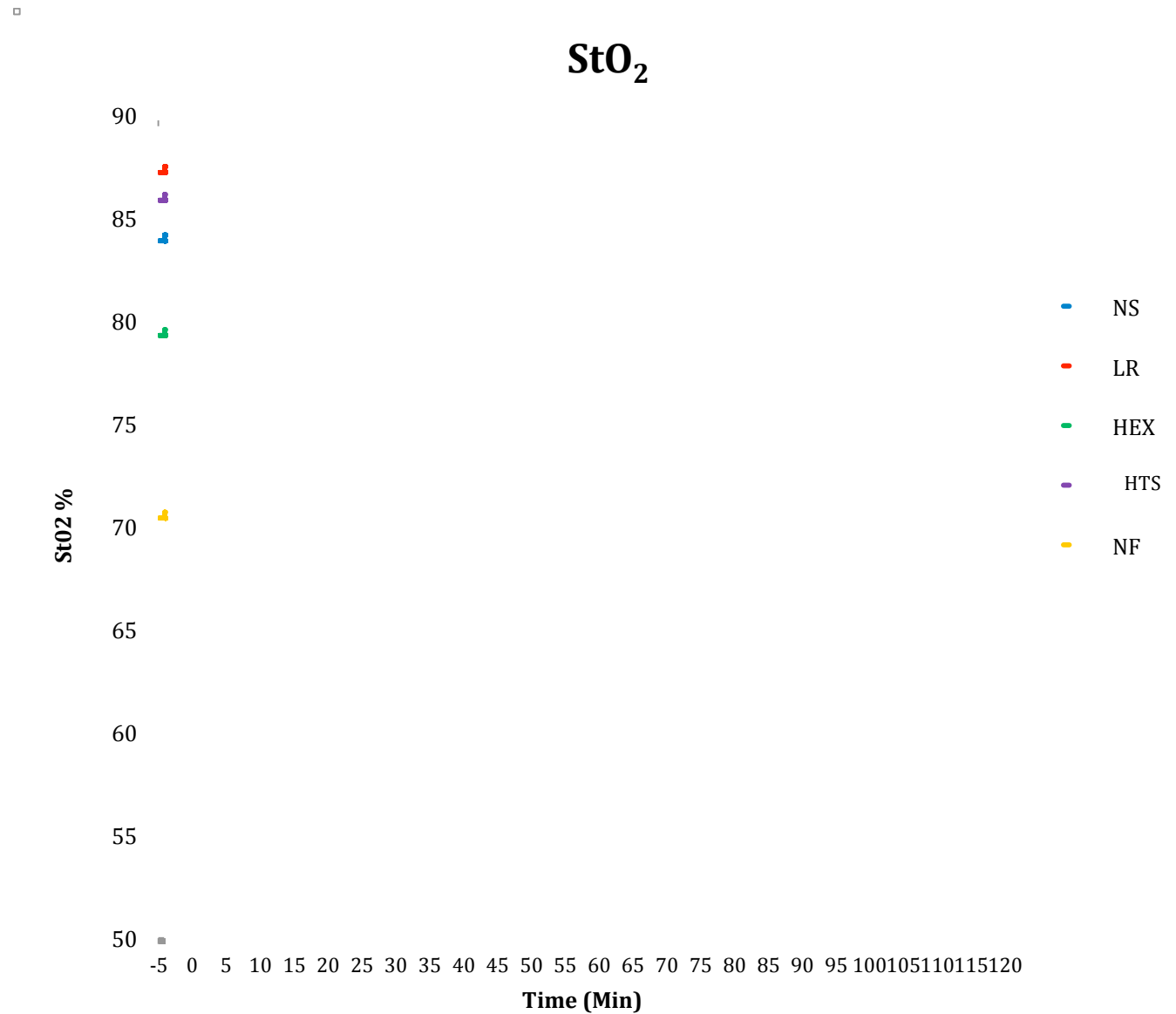

StO$_2$

Figure 4. Base excess data. Values presented as mean ± SEM. * indicates significantly lower
than HEX. Ŧ indicates significantly lower than HEX and LR. § indicates significantly
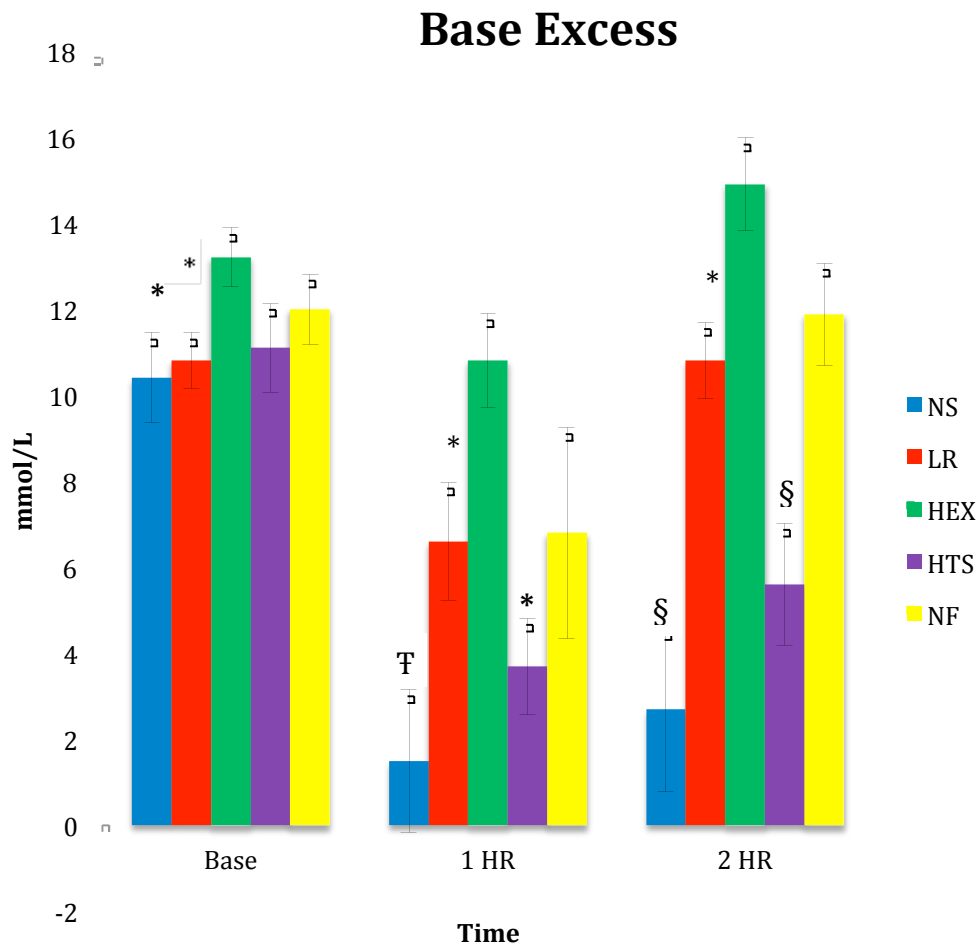lower than HEX, LR, and NF. For all significant comparisons, $p < 0.05$.

Table 1. Baseline weight, primary blood loss, survival, and secondary blood loss data.
Weight and primary blood loss presented as mean ± SEM. No significant differences in
weight, primary blood loss, or survival. Secondary blood loss presented as median with
IQR. [a] indicates significantly less than NS, LR, HEX, and HTS, $p < 0.01$.

Fluid	Weight	Primary blood loss	Survival	Secondary blood loss
NS	35.1 ± 0.6	1063.9 ± 103.9	10	138.5 (83.1, 169.6)
LR	34.5 ± 0.4	794.6 ± 82.3	10	127.5 (118.5, 134.5)
HEX	35.0 ± 0.6	870.5 ± 103.3	10	92.0 (77.4, 190.5)
HTS	35.9 ± 1.0	981.4 ± 126.5	10	99.1 (82.6, 145)
NF	34.2 ± 0.4	956.2 ± 139.9	8	62.3 (37.3, 80.1)[a]

Table 2. Hematocrit (Hct) data.

Values presented as mean ± SEM. [a] indicates significantly greater than NS, LR, HEX, and HTS. [b] indicates significantly greater than NS, HEX, and HTS. [c] indicates significantly greater than HEX and HTS. [d] indicates significantly greater than HEX. For all significant comparisons, $p < 0.03$.

Fluid	Base	Hct 1 HR	2 HR
NS	30.1 ± 0.7	20.0 ± 1.0	23.8 ± 0.8[d]
LR	29.8 ± 0.6	23.4 ± 0.8[b]	26.7 ± 1.2[c]
HEX	28.9 ± 0.7	20.6 ± 0.7	21.1 ± 0.7
HTS	30.0 ± 0.7	20.9 ± 0.5	23.3 ± 0.8
NF	29.5 ± 0.8	32.0 ± 0.9[a]	32.6 ± 1.0[a]

Table 3. MAP and StO_2 data at baseline, 1 hour, and 2 hours.

Values presented as mean ± SEM. [a] indicates significantly less than LR, HEX, and HTS. [b] indicates significantly less than NS, LR, HEX, and HTS. [c] indicates significantly less than HTS. For all significant comparisons, $p < 0.05$.

Fluid	MAP			StO_2		
	Base	1 HR	2 HR	Base	1 HR	2 HR
NS	68.5 ± 4.4	50.1± 2.9[a]	43.3 ± 2.7[a]	76.3 ± 2.7	76.8 ± 3.7	69.7 ± 2.5 [c]
LR	73.2 ± 4.5	63.3 ± 4.4	54.5 ± 3.4	75.8 ± 2.1	83.7 ± 2.1	74.5 ± 2.1
HEX	70.7 ± 4.1	62.8 ± 2.1	54.6 ± 2.5	72.4 ± 1.6	78.5 ± 3.2	77.8 ± 3.4
HTS	69.0 ± 2.9	60.0 ± 3.0	51.1 ± 2.2	73.6 ± 1.7	79.9 ± 1.0	77.1 ± 1.6
NF	71.4 ± 3.3	47.5 ± 4.8[a]	46.6 ± 5.3	69.7 ± 3.6	59.6 ± 5.6[b]	67.3 ± 3.2[a]

Table 4. pH and lactate data at baseline, 1 hour, and 2 hours.

Values presented as median with IQR. [a] indicates significantly less than LR, HEX, and NF. [b] indicates significantly less than LR and HEX. * indicates a significant increase in lactate from baseline to 1 hour in each respective group. [Ŧ] indicates a significant decrease in lactate from 1 hour to 2 hours in each respective group. There were no significant differences in lactate between groups. For all significant comparisons, $p < 0.05$.

	pH			Lactate		
Fluid	Base	1 HR	2 HR	Base	1 HR	2 HR
NS	7.54 (7.48, 7.59)	7.43 (7.36, 7.49)	7.43 (7.35, 7.51)[a]	1.9 (1.4, 2.3)	3.2 (2.5, 6.6)*	2.3 (1.8, 5.9)
LR	7.54 (7.51, 7.58)	7.47 (7.42, 7.53)	7.54 (7.49, 7.57)	1.8 (1.6, 2.4)	4.7 (3.3, 6.3)*	3.3 (2.1, 3.8)[Ŧ]
HEX	7.56 (7.53, 7.58)	7.51 (7.42, 7.56)	7.57 (7.50, 7.60)	1.4 (1.1, 2.2)	3.6 (2.7, 4.9)*	2.3 (2.0, 2.9)[Ŧ]
HTS	7.53 (7.46, 7.59)	7.44 (7.36, 7.48)	7.48 (7.37, 7.53)[b]	2.0 (1.5, 2.6)	3.6 (2.7, 4.9)*	2.8 (2.0, 3.9)[Ŧ]
NF	7.56 (7.50, 7.58)	7.49 (7.42, 7.53)	7.52 (7.50, 7.54)	2.2 (1.7, 2.5)	3.4 (2.7, 7.1)*	2.6 (2.4, 3.5)[Ŧ]